

10/729,427

- 14 -

REMARKSClaim Amendment

In the Office Action mailed on June 8, 2005, the Examiner issued a Restriction Requirement and a requirement for the election of species for the purposes of searching.

In response to the above requirements, Applicants elected the invention of Group I, covered by Claims 1-24 drawn to methods of treating a condition mediated by a proinflammatory cytokine. Within claims of Group I, Applicants elected chemical genera of subgroup 3 (anabaseine derivatives) for the purposes of searching. Claims readable on the elected genera are Claims 1-4, 10-15 and 20-24. Within claims of Group I, subgroup 3, Applicants elected diseases of class A (inflammatory conditions) and the species of rheumatoid arthritis for the purposes of searching.

In view of the expected withdrawal of the rejection of the generic Claim 1, it is requested that Claims 5-9, 16-19 and 24 be rejoined.

Claims 1 and 14 have been amended to correct typographical errors.

These amendments introduce no new matter.

Examiner's Interview

An Examiner's interview took place in the Offices of the USPTO on May 3, 2006. Applicants were represented by Steven Davis, Esq. Examiners M. Graffeo and A. Marschel represented the USPTO.

In the course of the interview, the parties discussed the rejection of claims under 35 U.S.C. §103(a) in view of the reference by Meyer (U.S. Pat. No. 5,977,144) and Borovikova (Borovikova *et al.*, "Vagus Nerve Stimulation Attenuates the Systemic Inflammatory Response to Endotoxin", *Let. to Nature* (2000) 405:458-462). The Examiner found the arguments for non-obviousness of the claimed invention presented by Applicants persuasive. Accordingly, Applicants request that the current claim rejections will be withdrawn.

Below, Applicants summarize the arguments presented during the interview.

10/729,427

- 15 -

Applicants Invention

The instant invention is based on the discovery that, from among the general class of α -bungarotoxin-sensitive receptors, it is stimulation of the $\alpha 7$ cholinergic receptor that alleviates inflammatory responses in a number of conditions. Prior to Applicants' discovery, the identity of the α -bungarotoxin-sensitive cholinergic receptor responsible for such alleviation was unknown. This discovery enabled Applicants to develop therapeutically effective methods of treating patients with inflammatory disorders by utilizing $\alpha 7$ -specific agonists without eliciting undesirable effects resulting from activation of other α -bungarotoxin-sensitive cholinergic receptors. Furthermore, because the identity of the α -bungarotoxin-sensitive cholinergic receptor involved in the inflammation was unknown until the Applicants' discovery, there was, correspondingly, no teaching or suggestion of selecting an agonist selective for an $\alpha 7$ nicotinic receptor for treating a patient suffering from inflammatory conditions as recited in Claim 1.

To support the above assertions, Applicants refer to Exhibit A, "MBC 3320 Acetylcholine," introduced with the Amendment of November 4, 2005, cholinergic receptors can be divided into two types, muscarinic and nicotinic. (Exhibit A, page 1, 3rd paragraph.) Nicotinic receptors are pentamers. (Exhibit A, page 2, 2nd paragraph.) There are five classes of subunits: α , β , γ , δ , and ϵ . Within each class of subunit, multiple types exist. Thus, there are nine known α -subunit types, four known β -subunit types, etc. The subunit composition of nicotinic receptors varies among tissue types (Exhibit A, page 2, last paragraph). α -Bungarotoxin binds to various types of both α and β subunits (Exhibit A, page 2, 3rd paragraph and page 3, the Table). Accordingly, without more one cannot tell which subunits make up an α -bungarotoxin-sensitive receptor.

Furthermore, there are many α -bungarotoxin-sensitive cholinergic receptors. To support this assertion, Applicants submit herewith the following exhibits:

- Exhibit B, a PubMed abstract of a publication by Johnson *et al.*, Mol. Pharmacol. (1995) Aug; 48(2):194-9, which teaches that α -bungarotoxin has high affinity not only for $\alpha 7$, but also for $\alpha 9$, $\alpha 1$ and other subtypes of nicotinic receptors.

10/729,427

- 16 -

- Exhibit C, a PubMed abstract of a publication by Anand *et al.*, Mol. Pharmacol. (1993) Nov; 44(5):1046-50, which teaches that at least three subtypes of α -bungarotoxin-sensitive nicotinic receptors exist, including $\alpha 8$.
- Exhibit D, a PubMed abstract of a publication by Pugh *et al.*, Mol. Pharmacol. (1995) Apr; 47(4):717-25, which teaches that α -bungarotoxin binds neuronal acetylcholine receptors that contain not only $\alpha 7$, but also $\alpha 9$, and $\alpha 1$ subunits.
- Exhibit E, a PubMed abstract of a publication by Colquhoun *et al.*, Adv. Pharmacol. (1997); 39:191-220, which teaches that α -bungarotoxin blocks activities of not only $\alpha 7$, but also $\alpha 8$, $\alpha 9$ and $\beta 2$ subunits of nicotinic receptors.
- Exhibit F, a commentary by Claude Libert on the publication by Wang *et al.*, Nature, vol. 421, 23 January 2003. The publication by Wang *et al.* forms the foundation of the present invention. Dr. Libert notes that, until the present discovery by Wang and Tracey (*i.e.* until the present invention), the precise identity of the nicotinic receptors responsible for the connection between the nervous system and inflammatory responses remained unknown and that "[i]t is difficult to find out, as the receptors are pentamers containing different combinations of a possible 16 monomers." (page 328, right column, last paragraph). As such, prior to the filing of the instant application, the skilled person did not know which nicotinic subunit or combination of different nicotinic subunits made up the α -bungarotoxin-sensitive cholinergic receptor that was responsible for mediating the inflammatory response.

Claims 1-24 are Non-obvious in View of Borovikova and Meyer

In the Office Action dated December 21, 2005, the Examiner stated that Borovikova teaches that α -bungarotoxin-sensitive nicotinic acetylcholine receptors are required for inhibition of TNF-mediated response. The Examiner further stated that one skilled in the art looking to agonize the $\alpha 7$ nicotinic receptors would be motivated to look to popular and known $\alpha 7$ -specific agonists such as those disclosed in Meyer.

The Borovikova reference, alone or in combination with other cited art, fails to identify the $\alpha 7$ cholinergic receptor as the relevant receptor subtype from among α -bungarotoxin-sensitive receptors generally, and therefore fails to motivate one skilled in the art to select $\alpha 7$ -

10/729,427

- 17 -

specific agonists to reduce inflammation. Although the Meyer reference discloses certain $\alpha 7$ -specific agonists, nothing in Meyer *et al.* teaches or suggests that the $\alpha 7$ -specific agonists disclosed therein reduce inflammatory responses and therefore can be used for treating inflammatory conditions. Because multiple classes of nicotinic acetylcholine receptors are α -bungarotoxin-sensitive, mere disclosure in the Meyer reference of certain $\alpha 7$ -specific agonists is insufficient to motivate one skilled in the art to $\alpha 7$ -selective agonists or $\alpha 7$ -specific agonists for treating an inflammatory condition without knowledge that activation of $\alpha 7$ cholinergic receptors can alleviate inflammatory responses.

Reconsideration and withdrawal of the rejection are respectfully requested.

Double Patenting Rejections

Applicants refer to M.P.E.P. §804(II)(B)(1)(a), which states:

If the application at issue is the later filed application or both are filed on the same day, only a one-way determination of obviousness is needed in resolving the issue of double patenting, i.e., whether the invention defined in a claim in the application is an obvious variation of the invention defined in a claim in the patent.

Since the application at issue was filed later than either U.S. Pat. No. 6,838,471 or U.S. Pat. No. 6,610,713, M.P.E.P. §804(II)(B)(1)(a) applies.

(1) Rejection of Claims 1-4, 10-15 and 20-23 Under Obviousness-type Double Patenting Doctrine over Claims 1-8, 11-14 and 18 of U.S. Pat. No. 6,838,471

Claims 1-4, 10-15 and 20-23 are rejected Under Obviousness-type Double Patenting Doctrine over Claims 1-8, 11-14 and 18 of U.S. Pat. No. 6,838,471 to Tracey (hereinafter, "Tracey I") in view of U.S. Pat. No. 5,977,144 to Meyer *et al.*

Similar to the art relied upon by the Examiner in advancing the rejection under 35 U.S.C. §103(a), nothing in the referenced claims of Tracey I teaches or suggests that from among α -bungarotoxin-sensitive receptors generally, it is the $\alpha 7$ nicotinic receptor subtype that can inhibit inflammatory responses, as recited in Claim 1 of the instant application. As discussed above, the Meyer reference also fails to teach or suggest that it is activation of the $\alpha 7$ receptors that alleviates disorders recited in Claim 1 of the present application.

10/729,427

- 18 -

Reconsideration and withdrawal of the rejection are respectfully requested.

(2) Rejection of Claims 1-4, 10-15 and 20-23 Under Obviousness-type Double Patenting Doctrine over Claims 1-3, 5-6, 14 and 16 of U.S. Pat. No. 6,610,713

Claims 1-4, 10-15 and 20-23 are rejected Under Obviousness-type Double Patenting Doctrine over Claims 1-3, 5-6, 14 and 16 of U.S. Pat. No. 6,610,713 to Tracey (hereinafter, "Tracey II") in view of U.S. Pat. No. 5,977,144 to Meyer *et al.*

Nothing in the referenced claims of Tracey II directs one skilled in the art toward an agonist selective for $\alpha 7$ receptors for treating inflammation. As discussed above, the Meyer reference also fails to teach or suggest that it is activation of the $\alpha 7$ receptors that alleviates disorders recited in Claim 1 of the present application.

Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Alexander Akhiezer

Alexander Akhiezer

Registration No. 54,617

Telephone: (978) 341-0036

Facsimile: (978) 341-0136

Concord, MA 01742-9133

Dated:

5/19/06



A service of the National Library of Medicine
and the National Institutes of Health

www.pubmed.gov

My NCBI
[Sign In] [Regs]

All Databases

PubMed

Nucleotide

Protein

Genome

Structure

OMIM

PMC

Journals

Books

Search PubMed

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display Abstract

Show: 20

Sort by

Send to

All: 1 Review: 0

About Entrez
NCBI ToolBar

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorials
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI

Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

☐ 1: Mol Pharmacol. 1995
Aug;48(2):194-9.

Related Articles, Compound via MeSH, Substance via MeSH,
Protein, Structure, 3D Domains, Cited in PMC, Books, LinkOut

Full text article at
www.molpharm.org

**alpha-Conotoxin ImI exhibits subtype-specific nicotinic
acetylcholine receptor blockade: preferential inhibition of
homomeric alpha 7 and alpha 9 receptors.**

Johnson DS, Martinez J, Elgoyhen AB, Heinemann SF, McIntosh JM.

Molecular Neurobiology Laboratory, Salk Institute, La Jolla, California
92037, USA.

Through a study of cloned nicotinic receptors expressed in *Xenopus* oocytes, we provide evidence that alpha-conotoxin ImI, a peptide marine snail toxin that induces seizures in rodents, selectively blocks subtypes of nicotinic acetylcholine receptors. alpha-Conotoxin ImI blocks homomeric alpha 7 nicotinic receptors with the highest apparent affinity and homomeric alpha 9 receptors with 8-fold lower affinity. This toxin has no effect on receptors composed of alpha 2 beta 2, alpha 3 beta 2, alpha 4 beta 2, alpha 2 beta 4, alpha 3 beta 4, or alpha 4 beta 4 subunit combinations. In contrast to alpha-bungarotoxin, which has high affinity for alpha 7, alpha 9, and alpha 1 beta 1 gamma delta receptors, alpha-conotoxin ImI has low affinity for the muscle nAChR. Related Conus peptides, alpha-conotoxins MI and GI, exhibit a distinct specificity, strictly targeting the muscle subtype receptor but not alpha 7 or alpha 9 receptors. alpha-Conotoxins thus represent selective tools for the study of neuronal nicotinic acetylcholine receptors.

PMID: 7651351 [PubMed - indexed for MEDLINE]

Display Abstract

Show: 20

Sort by

Send to

Write to the Help Desk

NCBI | NLM | NIH

Department of Health & Human Services

Privacy Statement | Freedom of Information Act | Disclaimer

☒ National Center for
Biotechnology
Information (NCBI)My NCBI ☒[\[Sign In\]](#) [\[Register\]](#)

All Databases

PubMed

Nucleotide

Protein

Genome

Structure

OMIM

PMC

Journals

Books

Search PubMed

for

☐ Limits☐ Preview/Index☐ History☐ Clipboard☐ Details

Display Abstract

Show 20

Sort by

Send to

[About Entrez](#)
[NCBI Toolbars](#)

All: 1

Review: 0

Text Version

[Entrez PubMed](#)
[Overview](#)[Help](#) | [FAQ](#)[Tutorials](#)[New/Noteworthy](#)[Utilities](#)[PubMed Services](#)[Journals Database](#)[PubMed Database](#)[Single Citation](#)[Matcher](#)[Batch Citation](#)[Matcher](#)[Clinical Queries](#)[Special Queries](#)[LinkOut](#)[My NCBI](#)[Related Resources](#)[Order Documents](#)[JLM Mobile](#)[JLM Catalog](#)[JLM Gateway](#)[TOXNET](#)[Consumer Health](#)[Clinical Alerts](#)[ClinicalTrials.gov](#)[PubMed Central](#)☐ 1: [Mol Pharmacol](#). 1993 Nov;44(5):1046-50.[Related Articles](#) [Links](#)☒ [Click here to read](#)**Pharmacological characterization of alpha-bungarotoxin-sensitive acetylcholine receptors immunoisolated from chick retina: contrasting properties of alpha 7 and alpha 8 subunit-containing subtypes.****Anand R, Peng X, Ballesta JJ, Lindstrom J.**

Department of Neuroscience, University of Pennsylvania, Philadelphia 19104-6074.

At least three subtypes of alpha-bungarotoxin-sensitive acetylcholine receptors (alpha Bgt-sensitive AChRs) exist in chick brain and retina. All may contain previously unknown structural subunits. One subtype contains alpha 7 subunits. Another contains alpha 8 subunits. A third contains both alpha 7 and alpha 8 subunits. In this article, we describe, for the first time, the pharmacological characterization of alpha 7 AChRs and alpha 8 AChRs immunoisolated from chick retina. Pharmacologically, the alpha 8 AChRs exhibit two classes of binding sites, the high affinity of which have higher affinity for most cholinergic ligands than do alpha 7 AChRs. These differences are most accentuated for ACh (approximately 5400-fold), decamethonium (approximately 1400-fold), 1,1-dimethyl-4-phenylpiperazinium (approximately 200-fold), atropine (approximately 200-fold), nicotine (approximately 100-fold), and tetramethylammonium (approximately 100-fold). The alpha 8 AChR low affinity sites exhibit affinities that are similar but not identical to that of alpha 7 AChRs. Many of the pharmacological differences between the alpha 7 AChRs and alpha 8 AChRs can be attributed to the limited differences between the amino acid sequences of the N-terminal region of the alpha 7 and alpha 8 subunits because expressed alpha 7 homomers and alpha 8 homomers also exhibit these characteristic differences.

PMID: 8246908; [PubMed - indexed for MEDLINE]

Display Abstract

Show 20

Sort by

Send to

[Write to the Help Desk](#)[NCBI](#) | [NLM](#) | [NIH](#)[Department of Health & Human Services](#)[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

Mar 22 2006 10:32:05

THIS PAGE BLANK (USPTO)

☐ National Center for
Biotechnology
Information (NCBI)

My NCBI

☐ [Sign In] [Register]

All Databases

PubMed

Nucleotide

Protein

Genome

Structure

OMIM

PMC

Journals

Books

Search PubMed

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display Abstract

Show 20

Sort by

Send to

About Entrez
NCBI Toolhar

All: 1 Review: 0

Text Version

Entrez PubMed
Overview

Help | FAQ

Tutorials

View Noteworthy

Articles

PubMed Services
Journals Database

MeSH Database

Single Citation

Matcher

Batch Citation

Matcher

Clinical Queries

Special Queries

LinkOut

My NCBI

Related Resources

Order Documents

JLM Mobile

JLM Catalog

JLM Gateway

TOXNET

Consumer Health

Clinical Alerts

Clinical Trials.gov

PubMed Central

☐ 1: Mol Pharmacol. 1995 Apr;47(4):717-25.[Related Articles](#), [Links](#)☐ Click here to read**Novel subpopulation of neuronal acetylcholine receptors among those binding alpha-bungarotoxin.****Pugh PC, Corriveau RA, Conroy WG, Berg DK.**

Department of Biology, University of California, San Diego, La Jolla 92093-0357, USA.

Neuronal acetylcholine receptors (AChRs) that bind alpha-bungarotoxin (alpha Bgt) (alpha Bgt-AChRs) have previously been found to contain at least one of the alpha 7-alpha 9 gene products. No other gene products of the 11 neuronal AChR genes cloned to date from rat and/or chick have been identified in such receptors. Chick ciliary ganglia have about 20 fmol of alpha Bgt-AChRs that contain alpha 7 subunits and 5 fmol of synaptic-type AChRs that bind the monoclonal antibody (mAb) 35 and collectively contain alpha 3, beta 4, alpha 5, and, to a lesser extent, beta 2 subunits. Using a sensitive solid-phase immunoprecipitation assay, we show here that ciliary ganglia have about 1 fmol of novel putative AChRs that bind both alpha Bgt and mAb 35 but appear to lack all of the known neuronal AChR gene products in ciliary ganglia, including alpha 3, alpha 5, alpha 7, beta 2, and beta 4. The putative receptors are also unlikely to contain either alpha 8 or alpha 9 gene products, because of the known expression patterns of these gene products. Nonetheless, the component sediments at 10 S, as expected for neuronal AChRs, and has a nicotinic pharmacology similar but not identical to that of alpha 7-containing alpha Bgt-AChRs. The AChR alpha 1 gene product expressed in muscle is known to bind both alpha Bgt and mAb 35, and we show here that ciliary ganglia contain small amounts of alpha 1 transcript. The putative ciliary ganglion AChR defined by joint alpha Bgt and mAb 35 binding, however, does not appear to contain alpha 1 subunits. A similar component binding both mAb 35 and alpha Bgt can be detected in sympathetic ganglia and dorsal root ganglia but not in brain, spinal cord, or retina. The developmental time course of the component in ciliary ganglia is comparable to that of the alpha 7-containing alpha Bgt-AChRs. If the component is a functional AChR on ciliary ganglion neurons, as seems likely, it would represent the fourth AChR subtype produced by this population of cells. Our inability to identify subunits comprising the putative receptors raises the possibility that additional AChR genes remain to be cloned.

PMID: 7723732 [PubMed - indexed for MEDLINE]

Display Abstract

Show 20

Sort by

Send to

[Write to the Help Desk](#)

[NCBI](#) | [NLM](#) | [NIH](#)

[Department of Health & Human Services](#)

[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

Mar 22 2006 06:32:05

National Center for
Biotechnology
Information (NCBI)

My NCBI

[Sign In] [Register]

All Databases
Search PubMed

PubMed

Nucleotide

Protein

Genome

Structure

OMIM

PMC

Journals

Books

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

Display Abstract

Show: 20

Sort by

Send to

About Entrez
CBI Toolbar

All: 1 Review: 1

Entrez Version

Entrez PubMed
Overview
Help | FAQ
Tutorials
New/Noteworthy
Utilities

1: Adv Pharmacol 1997;39:191-220.

[Related Articles](#) [Links](#)**Pharmacology of neuronal nicotinic acetylcholine receptor subtypes.****Colquhoun LM, Patrick JW.**

Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030, USA.

PubMed Services
Journals Database
MESH Database
Logic Citation
Batch Citation
Batch Citation
Clinical Queries
Special Queries
LinkOut
My NCBIRelated Resources
Order Documents
NCBI Mobile
NCBI Catalog
NCBI Gateway
OXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

The search for the physiological function of nicotinic receptors on neurons in the brain began with their discovery. It was initially assumed that, as in ganglia and at the neuromuscular junction, nicotinic receptors would gate fast synaptic transmission in the brain. The best functional evidence now, however, points to a role in modifying the release of other transmitters. This does not preclude a postsynaptic role in transmission for nicotinic receptors in the brain, but attempts to locate such a synapse have not been successful. If fast nicotinic synapses are present in the brain, they are probably low in number and may be masked by other, more prevalent synapses (such as glutamatergic) so identification will not be easy. The extent of diversity of nicotinic receptors is substantial. At the molecular level this is reflected in the number of different genes that encode receptor subunits and the multiple possible combinations of subunits that function in expression systems. From the cellular level there is a broad diversity of properties of native receptors in neurons. Some useful pharmacological tools allow the limited identification of subunits in native receptors. For example, block by alpha-bungarotoxin identifies alpha 7, alpha 8, or alpha 9 subunits; activation of a receptor by cytisine indicates an alpha 7 or beta 4 subunit; and neuronal bungarotoxin block identifies a beta 2 subunit. Despite the clues to identity gained by careful use of these agents, we have not been able to identify all the components of any native receptor based on pharmacological properties assessed from expression studies. When both pharmacological and biophysical properties of a receptor are taken into consideration, none of the combinations tested in oocytes mimics native receptors exactly. The reason for this discrepancy has been debated at length; it is possible that oocytes do not faithfully manufacture neuronal nicotinic receptors. For example, they may not correctly modify the protein after translation or they may allow a combination of subunits that do not occur in vivo. Another possibility is that correct combinations of subunits have not yet been tested in oocytes. Data from immunoprecipitation experiments suggest that many receptors contain three or more different subunits. Results from further experiments injecting combinations of three or more subunits into oocytes may be enlightening. The diversity of receptors may allow targeting of subtypes to specific locations. Nicotinic receptors are located presynaptically, preterminally, and on the cell soma. The function of the nicotinic

receptors located on innervating axons is presumably to modify the release of other neurotransmitters. It is an attractive hypothesis that nicotinic receptors might be involved in modifying the weight of central synapses; however, in none of the regions where this phenomenon has been described is there any evidence for axoaxonal contacts. The presynaptic receptors described so far are pharmacologically unique; therefore, if there are different subtypes of nicotinic receptors modifying the release of different transmitters, they may provide a means of exogenously modifying the release of a particular transmitter with drugs. There are still many basic unanswered questions about nicotinic receptors in the brain. What are the compositions of native nicotinic receptors? What is their purpose on neurons? Although there is clearly a role presynaptically, what is the function of those located on the soma? Neuronal nicotinic receptors are highly permeable to calcium, unlike muscle nicotinic receptors, and this may have important implications for roles in synaptic plasticity and development. Finally, why is there such diversity? (ABSTRACT TRUNCATED)

Publication Types:

- [Review](#)

PMID: 9160116 [PubMed - indexed for MEDLINE]

[Display Abstract](#)

Show 20 [Sort by](#) [Send to](#)

[Write to the Help Desk](#)

[NCBI](#) | [NLM](#) | [NIH](#)

[Department of Health & Human Services](#)

[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

Mar 22 2006 06:32:05

news and views

Inflammation

A nervous connection

Claude Libert

The molecular details of a connection between the nervous system and the inflammatory response to disease have been uncovered. This suggests new avenues of research into controlling excessive inflammation.

Sepsis is a complex, exaggerated and chaotic version of the usually well-organized inflammatory arm of our immune defences, and kills over 175,000 people each year in the United States alone¹. Although a great deal of time and effort has been spent researching septic shock, it remains difficult to understand and treat. One promising lead was provided two years ago, when it was discovered that there is a connection between inflammation and the involuntary nervous system. The details of this link have, however, been unclear — until now. Writing on page 384 of this issue, Kevin Tracey and colleagues² describe how they identified a receptor protein that is stimulated by the nervous system and which in turn inhibits a key molecular mediator of inflammation and septic shock. This receptor might make a good target for future drugs to treat sepsis.

Inflammation has several roles in the body, one of which is to contribute to the immune system's ability to fight off invading microorganisms. For instance, molecules that are produced during the inflammatory response increase blood flow to infected areas, or help to recruit immune cells. One way in which inflammation is triggered is in response to lipopolysaccharides — components of the cell walls of many bacteria — which activate the immune system's macrophages. These cells in turn release 'alarm' molecules, namely cytokines, some of which have powerful pro-inflammatory properties. Tumour-necrosis factor (TNF) is one such molecule. This protein can affect nearly all cell types, and has a range of biological activities. For instance, it induces the expression of a large number of genes that encode essential inflammatory molecules (such as other cytokines; enzymes that help to break down the barriers between cells, allowing the migration of immune cells, and adhesion molecules that again enhance immune-cell migration)^{3,4}.

As long as TNF production remains confined to the site of infection, the inflammatory response is clearly beneficial. But once bacteria, and consequently TNF, invade the systemic blood circulation, blood 'poisoning' and sepsis can develop quickly. Furthermore, TNF has been found to be a central mediator of chronic inflammatory disorders such as rheumatoid arthritis and Crohn's disease. So there is much interest in learning how to control the production, release and

activity of TNF. Several means of doing so have been developed (Fig. 1), and have seen some success in treating certain inflammatory disorders⁵. For instance, there are drugs that inhibit the transcription of the TNF-encoding gene into messenger RNA, the translation of the mRNA into protein, or the release of the TNF protein. There are also antibodies and soluble receptors that bind to and block TNF once it has been released. But, although the value of these approaches is beyond doubt, they all take time to work — and time is usually short when treating patients with sepsis.

Tracey's research team has been studying TNF since this protein was discovered (see, for instance, ref. 6). Recently, Tracey's group described another level of control of TNF synthesis — namely by means of the vagus nerve⁷ — thereby providing a new and exciting link between the involuntary nervous system and inflammation. This 'parasympathetic' nerve emanates from the cranium and innervates all major organs in a subcon-

scious way. It is finely branched and is composed of both sensory (input) and motor (output) fibres. This is of relevance because it means that the vagus nerve can on the one hand sense continuing inflammation (presumably by detecting cytokines through receptors on the nerve surface), and on the other hand suppress it. This suppression is efficient and, above all, a good deal faster than the mechanisms mentioned above. Tracey's group found⁷ that, after injecting lipopolysaccharides into rats, electrically stimulating the vagus nerve prevented both the release of TNF from macrophages, and death. Conversely, surgically severing the nerve not only removed this protection but also sensitized the animals to lipopolysaccharide.

But how does the vagus nerve have this effect on macrophages? It was already known that, after this nerve is stimulated, its endings release the neurotransmitter molecule acetylcholine with lightning speed. Macrophages express acetylcholine receptors known as nicotinic receptors, and respond to the released acetylcholine (or the acetylcholine-mimicking nicotine), by suppressing TNF release. But the precise identity of the nicotinic receptors on macrophages was not known. From a therapeutic point of view, this is clearly important to know. It's also very difficult to find out, as the receptors are pentamers containing different combinations of a possible 16 monomers.

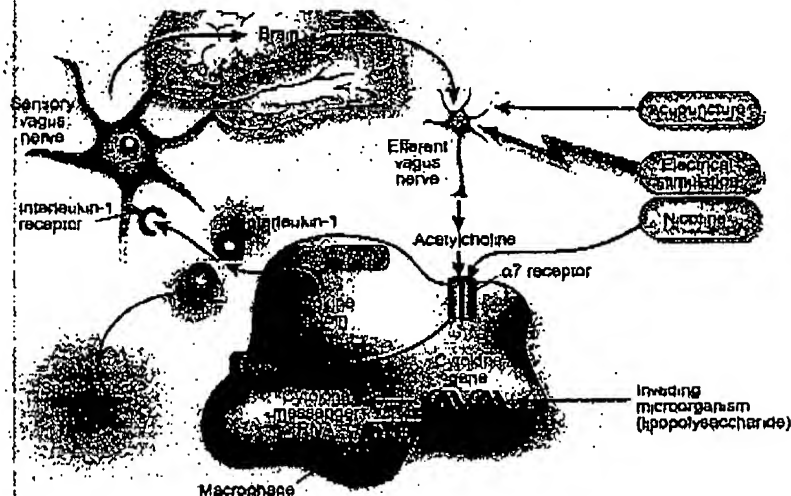


Figure 1 The inflammatory response to microorganisms, and ways of controlling it. Clockwise from lower right: many bacteria contain lipopolysaccharide in their cell walls, which stimulates macrophages. These immune cells then make and release various cytokines ('alarm') molecules, including tumour-necrosis factor (TNF) and interleukin-1. But too much TNF in the blood can be harmful, leading to excessive inflammation and septic shock. Several drugs (orange boxes) inhibit steps in TNF synthesis. In addition, Tracey and colleagues have found that when the vagus nerve detects interleukin-1 (left), it releases acetylcholine (right), which binds to the $\alpha 7$ receptor⁷ on macrophages and inhibits cytokine production. This suggests possible new ways of controlling inflammation: through electrically stimulating the vagus nerve, by acupuncture, or with the use of nicotine (which mimics acetylcholine).

news and views

In their latest paper, Tracey and colleagues¹ put down the relevant nicotinic acetylcholine receptor: it is one comprising five copies of the monomer $\alpha 7$. They started by using α -bungarotoxin, a molecule that binds to just a subset of receptor monomers, to show that macrophages express the $\alpha 7$ subunit. When the authors blocked the expression of this protein, acetylcholine and nicotine were no longer able to prevent the release of TNF — data that the authors confirmed by studying $\alpha 7$ -deficient mice. In fact, such mutant mice displayed an exaggerated response to lipopolysaccharide in terms of their production of the cytokines TNF, interleukin-1 and interleukin-6. Finally, in a technical tour de force, Tracey and colleagues showed that electrically stimulating the vagus nerve of $\alpha 7$ -deficient mice no longer afforded protection against lipopolysaccharide (in contrast to the situation in wild-type mice).

These findings¹ could have therapeutic implications. The discovery of the connection between the involuntary nervous system and inflammation had already yielded new ideas about treating inflammatory disorders such as sepsis; for instance, a small compound has been developed that can trigger the vagus nerve in rats, thereby reducing inflammation². Looking to the future, it would be interesting to stimulate the vagus nerve electrically in people — as is currently done in thousands of epilepsy patients, showing that the procedure is safe and feasible — and to study the effect on inflammation. More specifically, the new findings suggest that molecules that stimulate the $\alpha 7$ subunit would also be worth developing.

On a different note, nicotine has been found to have powerful immunosuppressive and inflammation-suppressing effects. Of course, the health risks associated with smoking are immense. Yet epidemiological studies indicate that nicotine protects against several inflammatory diseases, such as ulcerative colitis, Parkinson's disease and even Alzheimer's disease. It can also reduce fever and protect against otherwise lethal infection with the influenza virus³. The demonstration¹ that nicotine binds to the $\alpha 7$ subunit on macrophages fleshes out the details of how nicotine produces such effects.

The data also make me reconsider the possibilities and molecular biology of 'alternative' medicine. Pavlovian-type conditioning, hypnosis and meditation are well known (since the beginning of the twentieth century in some cases) to reduce inflammation⁴. It might be worth finding out whether these effects, as well as the reported beneficial effects of prayer and acupuncture on inflammation (the last of which is known to depend on acetylcholine)^{5,6}, are mediated by the vagus nerve and the $\alpha 7$ subunit.

Claude Libert is in the VIB/RUG Department of

Molecular Biomedical Research, Ledeganckstraat 35, Ghent 9000, Belgium

e-mail: claude.libert@vib.rug.ac.be

1. Scow R. *Science* 64, 460–467 (1994).
2. Wang, H. et al. *Nature* 421, 384–388 (2002), and references therein.
3. Viscidi P. *Ann. Rev. Immunol.* 10, 411–432 (1992).
4. Winkler, B. et al. *Nature Med.* 7, 1202–1208 (2001).

5. Fukumura, M. *Nature Rev. Immunol.* 2, 369–371 (2002).
6. Truax, K. J. et al. *Science* 234, 470–474 (1986).
7. Borovikova, L. V. *Nature* 403, 436–437 (2000).
8. Burch, T. R. et al. *J. Exp. Med.* 195, 781–788 (2002).
9. Saper, M. *Nature Rev. Immunol.* 2, 372–377 (2002).
10. Talley, N. J. & Spiller, R. *Lancet* 360, 555–564 (2002).
11. Sun, Y. S. et al. *Neurosci. Lett.* 318, 42–48 (2002).
12. King, D. E., McIntosh, A. C. III, Saper, T. E. & Parnham, W. *Int. J. Psychopharmacol.* 11, 415–425 (2001).

Astronomy

Feeding the first quasars

Laura Ferrarese

Quasars, the oldest known objects in the Universe, are powered by gas falling into black holes at their centres. How black holes formed so early in time has been hard to explain, but a new model might have the answer.

The excitement that has, in recent years, accompanied the study of supermassive black holes mirrors the excitement that followed the discovery of quasars in the early 1960s. Quasars — short for 'quasi-stellar objects' — are characterized by a prodigious outpouring of energy: hundreds of times that of a regular galaxy, but produced in a region that is only a few light days or weeks across. Quasars are also among the most distant objects known to astronomers; as such, the light reaching the Earth from them paints an invaluable picture of the history of our Universe. Supermassive black holes have long been accepted as the only viable energy source for quasars, but only now are we beginning to understand the complex connection between black holes and the formation of galaxies.

Quasars are thought to reside at the centres of massive haloes of dark matter — the mysterious, unseen matter that is needed to explain many features of our Universe. Indeed, this is a crucial assumption that underlies some of the most popular models of black-hole formation. On page 341 of this issue, Barkana and Loeb¹ suggest that if a dark-matter halo is pulling gas towards a quasar at its centre, a distinctive signature should be seen in the light reaching us from the quasar. There are few data to go on so far, but if this signature is confirmed, it would provide the first observational evidence that quasars are embedded in great haloes of dark matter.

In the local Universe, there is now airtight evidence for the presence of supermassive black holes in two galaxies — the Milky Way^{2,3} and the nearby Seyfert II galaxy, NGC 4258 (ref. 4). Compelling, although more indirect, evidence of black holes exists for at least two dozen additional galaxies⁵. These supermassive black holes have masses that range from a few million to a few billion times that of our Sun. Their host galaxies are sufficiently disparate in nature that it is now possible to search for connections between their large-scale properties and the masses of

their central black holes. Some connections have already been identified, most notably between supermassive black-hole masses and the velocity distribution of the surrounding hot stellar component^{6,7}. Another connection may exist between the mass of the central black hole and the mass of the surrounding dark-matter halo⁸.

The demographics of more distant supermassive black holes can be inferred from a census of quasars. In the past few years, the Sloan Digital Sky Survey (SDSS) has dramatically increased the number of known quasars at 'high redshift', where redshift is a measure of an object's recession velocity due to the expansion of the Universe. The SDSS^{9–11} has found several quasars with redshifts larger than 5, including the current record holder at a redshift of 6.43. Assuming that these high-redshift quasars are radiating at the Eddington limit (the maximum luminosity that can be sustained by accretion), their luminosities imply central black holes with masses at least several billion times that of the Sun.

It has been pointed out¹² that at a redshift of 5 we are looking back in time to when the age of the Universe (about 1 billion years) was approximately equal to the dynamical timescale of a typical galaxy — roughly speaking, the stellar orbital time, or the time it takes a galaxy to communicate with itself through its own gravitational potential. Thus, the very existence of quasars at such high redshifts is a challenge to models of structure formation^{13,14}. Although the details vary, the basic assumption underlying virtually all models is that, at all redshifts, the history of supermassive black holes follows the evolution of galactic dark-matter haloes. In particular, the black-hole mass is assumed to scale with halo mass, and black-hole growth proceeds through gas accretion triggered by galaxy mergers.

A relation between black-hole and dark-halo mass is a feature of models that account for supermassive black holes within this 'hierarchical' framework, in which structure

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☒ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.